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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Paper No. 21

Application Number: 09/578,361
Filing Date: May 24, 2000
Appellant(s): MAES ET AL.

Andrew F. Niles
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 6 December 2002 (hereinafter, the Brief).

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

On page 2, item (2) of the Brief, Appellants state that "Neither the appellant, the appellant's representative, nor the assignee is aware of any pending appeal or interference which would directly affect, be directly affected by or have any bearing on the Board's decision in the present pending appeal."

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

Appellant's brief includes a statement that claims of Groups I (claims 1-3, 7, 9-11, 20 and 22), Group II (claim 4), Group III (claims 5, 6, 8 and 12), Group IV (claims 13-17) and Group V (claims 19 and 21) do not stand or fall together.

Therefore, the following groups of claims will be considered:

Group I: Claims 1-3, 7, 9-11, 20 and 22

Claims 1-3, 7, 9-11, 20 and 22 do not stand or fall together with claims of Group II, Group III, Group IV, or Group V, as being rejected over the combination of Dellaporta and Koes et al.

Group II: Claim 4

Claim 4 does not stand or fall together with claims of Group I, Group III, Group IV or Group V, as being rejected over the combination of Dellaporta, Koes et al. and Sour et al.

Group III: Claims 5, 6, 8 and 12

Claims 5, 6, 8 and 12 do not stand or fall together with claims of Group I, Group II, Group IV or Group V, as being rejected over the combination of Dellaporta, Koes et al. and Vos et al.

Group IV: Claims 13-17

Claims 13-21 do not stand or fall together with claims of Group I, Group II, Group III or Group V, as being rejected over the combination of Dellaporta and Koes et al.

Group V: Claims 19 and 21

Claims 19 and 21 do not stand or fall together with claims of Group I, Group II, Group III or Group IV, as being rejected over the combination of Dellaporta and Koes et al.

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

6,013,486	DELLAPORTA	01-2000

Koes, R. et al., "Targeted gene inactivation in petunia by PCR-based selection of transposon insertion mutants" PNAS USA, vol. 92, pp. 8149-8153 (1995).

Souer, E. et al., "A general method to isolate genes tagged by a high copy number transposable element" The Plant Journal, vol. 7 (4), pp. 677-685 (1995).

Vos, P. et al., "AFLP: a new technique for DNA fingerprinting" Nucl. Acids Research, vol. 23 (21), pp. 4407-4414 (1995).

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims rejected under 35 U.S.C. 103(a). This rejection is set forth in prior Office Action, Paper No. 11.

Claims 1-3, 7, 9-11, 19, 20 and 22, under appeal, are rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta (U.S. Patent No. 6,013,486) and Koes et al. (PNAS USA, Vol. 92, pp. 8149-8153, 1995).

A) Dellaporta teaches a method of screening for gene insertion mutants in a population of organisms by preparing a library of insertion elements and insertion element flanking sequences, amplifying the insertion elements flanking sequences using primers derived from the flanking sequences, and either fixing the amplified products to a solid support to serve as targets for hybridization or the labeling the amplified products to be used as probes. The organism can be a plant. The amplification of insertion element flanking sequences can be achieved by iPCR (inverse PCR), which comprises digesting the insertion element mutant library with a restriction enzyme, self-ligation of fragments to form circles and amplifying the insertion element flanking sequences using primers based on the terminal part of the insertion element. The digesting

enzyme could be BfaI or MseI (col. 2, lines 1-15, 57-67; col. 3, lines 48-67; col. 4, lines 1-8, 39-46; col. 5, lines 15-34; col. 11, lines 43-67; col. 12, lines 1-39; col. 13, lines 58-67; col. 14, lines 1-5; col. 15, lines 6-14, 47-57; col. 28, lines 17-19).

Pools containing DNA from different combinations of individuals, designed in such a way that sequences representing single members of a population can be identified without the need to analyze each member individually. For example , pools can be distributed into a 2x2 grid, comprising rows and columns. (col. 3, lines 58-65; col. 15, lines 58-67).

B) Dellaporta teaches forming pools of DNA but does not teach 30 DNA samples from 100 plants each, wherein the DNA from 100 plants is distributed into a 3D array of 10 blocks, 10 rows and 10 columns.

C) Koes et al. describe a method of preparing an insertion element mutant library of transposable elements dTph1 in petunia plants. They describe pooling plant material from three sets of 1,000 plants each in patterns of blocks, rows and columns, e.g. 10 blocks, 10 rows, 12 columns (page 8150, col. 2, par. 4,5; Fig. 2, 3).

It would have been obvious to one of ordinary skill in the art at the time of the invention to have used the DNA pooling method of Koes et al. in the insertion element library screening method of Dellaporta. The motivation to do so, expressly provided by Koes et al., would have been that three-dimensional was less laborious (single round of screening), less liable to detect false positives and identified single plants directly.

Claim 4, under appeal, is rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta and Koes et al. as applied to claims 1-3 above, and further in view of Souer et al. (The Plant Journal, Vol. 7, pp. 677-685, 1995).

A) Claim 4 is drawn to reamplifying at least one amplifiable genomic fragment with at least one primer based on a sequence of a nucleic acid insertion element.

B) Neither Dellaporta nor Koes et al. teach reamplifying at least one amplifiable genomic fragment with at least one primer based on a sequence of a nucleic acid insertion element.

C) Souer et al. teach a method of isolating gene insertion mutants in petunia plants based on the amplification of insertion element dTph1 flanking sequences using a combination of iPCR and differential screening of amplification products (page 678, col. 1, par. 1). Amplification by iPCR comprises:

i) digesting genomic DNA using a restriction enzyme,

ii) self-ligation of the digested fragments to form circles, and

iii) amplification with an insertion element specific primer (page 678, col. 2, last paragraph; Fig. 2).

Amplification yield can be improved by using re-amplification with nested primers complementary to the insertion element (page 680, col. 1, par. 1).

It would have been obvious to one of ordinary skill in the art at the time of the invention to have used re-amplification of Souer et al. in the combined method of Dellaporta and Koes et al. The motivation to do so, expressly provided by Souer et al, would have been that re-amplification improved the yield of amplification of dTph1 flanking sequences.

Claims 5, 6, 8 and 12, under appeal, are rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta and Koes et al. as applied to claim 1 above, and further in view of Vos et al. (Nucleic Acids Res., Vol. 23, pp. 4407-4414, 1995).

A) Claims 5 and 6 are drawn to "transposon display amplification" comprising:

i) digesting nucleic acid sequences from the gene insertion mutant library with two restriction enzymes, one cutting a 6 base pair (bp) site and the other a 4 bp site,

ii) ligating a biotinylated adaptor to the hexacutter site and a second adaptor to the tetracutter site,

iii) selecting biotinylated restriction fragments using streptavidin beads,

iv) amplifying insertion element flanking sequences using primer based on the biotinylated adaptor and insertion element sequence and a primer complementary to the second adaptor,

v) re-amplifying insertion element flanking sequences using nested primer based on the insertion element and a primer complementary to the second adaptor.

Claim 8 is drawn to nucleic acid sequences selected from the group consisting of genomic DNA and cDNA, and claim 12 to using a restriction enzyme from the group consisting of MseI and MunI.

B) Neither Dellaporta nor Koes et al. teach amplification by transposon display amplification.

C) Vos et al. teach a DNA fingerprinting technique (transposon display amplification) comprising:

i) digesting DNA with two restriction enzymes, recognizing a 6 bp and 4 bp sites, e.g. EcorI and MseI,

ii) ligating a radiolabelled adaptor to the hexacutter site and a second adaptor to the MseI site,

iii) amplification of the restriction fragments using primers complementary to the adaptors and restriction site sequences.

The fragments could be subjected to a second round of amplification using modified primers (Abstract; page 1408). The amplified fragments can be selected by using a biotinylated adapter for the hexacutter site and separated from the rest of the fragments with streptavidin beads (page 4413, col. 2, par. 3).

It would have been obvious to one of ordinary skill in the art at the time of the invention to have used the DNA amplification method of Vos et al. with the library of gene insertion mutants of Dellaporta and Koes et al. The motivation to do so, expressly provided by Vos et al., would have been that amplification and isolation of DNA fragments was achieved without the prior knowledge of their sequences.

Claims 13-17 and 21, under appeal, are rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta and Koes et al. as applied to claims 1 and 19 above.

A) Claims 13-17 are drawn to a kit for performing the method of claim 1, comprising DNA samples of an insertion element mutant library, a set of amplified insertion element flanking sequences, which may be fixed to a solid support, be in soluble or dried state or be labeled with fluorescein. Claim 21 is drawn to a kit for performing the method of claim 19, the kit comprising DNA samples of an insertion element mutant library.

B) The reagent kits for performing DNA detection assays were conventional in the field of molecular biology at the time of the invention.

Therefore it would have been obvious to one of ordinary skill in the art at the time of the invention to have packaged the insertion element mutant library and amplified insertion element

flanking sequences into a kit for the expected benefits of convenience and cost-effectiveness for practitioners in the art wishing to perform screening for insertion mutants.

(11) Response to Argument

Group I (claims 1-3, 7, 9-11, 20 and 22): rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta in combination with Koes et al. (Final Office Action, Paper No. 11, mailed December 19, 2001).

Appellants arguments:

A) "Dellaporta does not teach or suggest an insertion element library built into a 3D-array of block, row and column pools as required by claim 1. Rather, Dellaporta is limited to a "2x2 grid, [where] pools of DNA are then prepared from all of the individuals within each column and row... [a]lternatively, pools needn't be used." (*Id.*, Col. 15, lines 66-67 and Col. 16, line 3)." (page 6).

B) Dellaporta does not teach or suggest "amplifying each of said plurality of insertion element flanking sequences from said block, row and column pools", as claimed by claim 1 (page 6, the last paragraph and page 7, the first paragraph). According to Appellants, Dellaporta teaches amplification methods which are limited to "using a single primer set [that] may amplify a representative sample of insertion junctions from a particular group of individuals" (Col. 12, lines 6-8), and amplification of a large number of insertional mutants such that there will be a high probability of identifying a mutant for any given locus within the population (Col. 3, lines 54-58).

C) Koes et al. do not teach "amplifying each of said plurality of insertion element flanking sequences from said block, row and column pools", as claimed by claim 1 (page 7, second paragraph).

D) Dellaporta and Koes et al., neither alone, nor in combination, teach all of the claim limitations of claim 1 (page 7, third paragraph).

E) Combination of Dellaporta and Koes et al. is improper, because there is no motivation to combine the teachings of these two references. "The motivation provided by the Examiner to combine Dellaporta and Koes et al. does not suggest a desirability for combining the references, but merely restates why Koes et al. prefers a "one-step three dimensional screening over three repeated rounds of one-dimensional screening." (page 8, the first paragraph).

F) Combination of Dellaporta and Koes et al. is improper because Dellaporta teaches away from Koes et al. (page 8, second and third paragraphs and page 13, second paragraph). Specifically, Appellants argue that Dellaporta discourages the use of teachings of Koes et al., since Koes et al. teach "site-selected" approach to identifying insertional mutations, and Dellaporta argues that "site-selected" mutagenesis has had limited success in applications to large-scale genomic investigations.

Summarizing, Appellants argue that neither Dellaporta nor Koes et al. or their combination teaches all of the elements of claim 1 and that combining of Dellaporta and Koes et al. is improper because there is no motivation to combine the references and Dellaporta teaches away from Koes et al.

Before proceeding with the arguments, a glossary of terms will be established to facilitate comparison of references with Appellants' claimed subject matter.

Appellants' term	Dellaporta's term
insertion element	insertional mutant
	insertional mutagen
	transposable element
insertion element flanking sequence	insertion junction
"site-selected"	gene-specific
adaptor	adapter

Response to Appellants' arguments

Teaching or suggesting of all the elements of claim 1. Regarding claim 1, Dellaporta teaches a method of simultaneous screening for gene insertion mutants in a population of organisms by preparing a library of insertion elements and insertion element flanking sequences, amplifying the insertion elements flanking sequences using primers derived from the flanking sequences, and fixing the amplified products to a solid support to serve as targets for hybridization. The organism can be a plant. (col. 2, lines 1-15, 57-67; col. 3, lines 48-67; col. 4, lines 1-8, 39-46; col. 5, lines 15-34; col. 13, lines 58-67; col. 14, lines 1-5; col. 15, lines 6-37).

Dellaporta teaches amplification of insertion element flanking sequences by iPCR (inverse PCR), which comprises digesting the insertion element mutant library with a restriction enzyme, self-ligation of the resulting amplifyable fragments to form circles and amplifying the insertion element flanking sequences using primers based on the terminal part of the insertion element (col. 11, lines 43-67; col. 12, lines 1-39).

Dellaporta teaches pools containing DNA from different combinations of individuals, designed in such a way that sequences representing single members of a population can be

identified without the need to analyze each member individually. For example , pools can be distributed into a 2x2 grid, comprising rows and columns. (col. 3, lines 58-65; col. 15, lines 58-67). Dellaporta does not specifically teach pooling individuals into 3D (three-dimensional) array of block, row and column pools.

Koes et al. teach a method of preparing an insertion element mutant library of transposable elements dTph1 in petunia plants. They describe pooling plant material from three sets of 1,000 plants each in patterns of blocks, rows and columns, e.g. 10 blocks, 10 rows, 12 columns (page 8150, col. 2, par. 4,5; Fig. 2, 3).

As to the limitation of amplifying each of the plurality of insertion element flanking sequences, Dellaporta teaches amplification by inverse PCR (iPCR) (see above), which is claimed by the Appellants as an embodiment which results in amplification of each of the insertion element flanking sequences (claims 2 and 3). Therefore it would be reasonable to assume that he would achieve this result because he uses exactly the same method of amplification as claimed by Appellants.

Therefore, Dellaporta and Koes et al. combined teach all of the elements of claim 1.

Dellaporta teaches away from Koes et al. Regarding the argument that Dellaporta teaches away from Koes et al. because Dellaporta considers gene-specific amplification of insertional junctions inefficient: in the rejection of claim 1, Koes et al. is relied on for the teaching of a 3D pooling technique, not for the amplification method, whereas Dellaporta is relied on for teaching of the amplification of insertion junctions.

Motivation to combine Dellaporta and Koes et al. Finally, regarding motivation to combine Dellaporta and Koes et al., Dellaporta teaches preparation of pools (= libraries) of

insertion junctions by pooling DNA from individuals, so that "the source of a particular insertion junction can be identified without the need for screening of all individuals within a population" (col. 15, lines 62-64). An exemplary pooling procedure identified by Dellaporta is a 2x2 grid, consisting of rows and columns, which enable precise identification of an individual sequence. As stated by Dellaporta "Alternatively, pools needn't be used, however, this will be less preferred as more effort will be needed to find a specific desired insertion." (col. 16, lines 3-5; emphasis added; the underlined part is a continuation of a sentence cited by Appellants in support of their argument that Dellaporta does not provide motivation for pooling). Therefore, Dellaporta advocates using pooling of individual sequences in order to efficiently locate a desired sequence.

Dellaporta does not teach pooling individuals into a 3D (three-dimensional) array of block, row and column. Koes et al. teach a method of preparing an insertion element mutant library of transposable elements dTph1 in petunia plants. They describe 3D pooling of plant material from three sets of 1,000 plants each in patterns of blocks, rows and columns, e.g. 10 blocks, 10 rows, 12 columns (page 8150, col. 2, par. 4,5; Fig. 2, 3). The motivation provided by Koes et al. for using the three-dimensional pooling method is "We prefer this one-step three-dimensional screening over three repeated rounds of one-dimensional screening, as employed in Fig. 1, because (i) it is less laborious; (ii) it is less liable to detect false positives due to somatic insertions of dTph1, as such sectors rarely expand to all the leaves of the plant and, thus, do not produce signals in all three dimensions of the screen (see Fig. 3); and (iii) it directly identifies a single plant." (page 8152, third paragraph).

Both Dellaporta and Koes et al. provide compelling motivation for using pooling of samples: efficient screening for desired sequences. Koes et al. provide additional motivation for using 3D pooling as being less prone to produce false positive signals and less laborious than one-dimensional screening.

Group II (claim 4): rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta in combination with Koes et al. and Souer et al. (Final Office Action, Paper No. 11, mailed December 19, 2001).

Appellants arguments:

A) Neither Dellaporta nor Koes et al. teach reamplifying at least one amplifyable genomic fragment of claim 3 (page 9, third paragraph).

B) Souer et al. do not teach preparing an insertion element mutant library built in a 3D array of block, row and column pools, and "none of the cited references teach the limitation of amplifying each of the plurality of insertion flanking sequences from the block, row and column pools of claim 1." (page 9, third paragraph).

C) The references do not suggest or provide a motivation for combining them. Appellants cite M.P.E.P. 2143.01, "the level of skill in the art cannot be relied upon to provide the suggestion to combine references." (page 9, the last paragraph).

Response to Appelants' arguments

Teachings of the references. Teachings of Dellaporta and Koes et al. were discussed above and were shown to provide all of the elements of claim 1. Dellaporta combined with Koes et al. also teaches claims 2 and 3, since Dellaporta teaches amplification of insertion element flanking sequences by iPCR (inverse PCR), which comprises digesting the insertion element

mutant library with a restriction enzyme, self-ligation of the resulting amplifyable fragments to form circles and amplifying the insertion element flanking sequences using primers based on the terminal part of the insertion element (col. 11, lines 43-67; col. 12, lines 1-39).

Souer et al. teach a method of isolating gene insertion mutants in petunia plants based on the amplification of insertion element dTph1 flanking sequences using a combination of iPCR and differential screening of amplification products (page 678, col. 1, par. 1). Amplification by iPCR comprises:

i) digesting genomic DNA using a restriction enzyme,

ii) self-ligation of the digested fragments to form circles, and

iii) amplification with an insertion element specific primer (page 678, col. 2, last paragraph; Fig. 2).

Amplification yield can be improved by using re-amplification with nested primers complementary to the insertion element (= based on a sequence of the insertion element) (page 680, col. 1, par. 1). Therefore, Souer et al. teach amplification of each of the insertion element flanking sequences, since they teach iPCR (see discussion for claims of Group I). The amplified flanking sequences are cloned and subsequently used as probes in screening of plant DNA for genes in which the insertion events occur (Abstract; page 678, first and last paragraphs; page 682, second paragraph). Therefore the yield of amplification and the specificity of amplification are important, since otherwise too little of the insertion element flanking sequence may be produced to be used in screening procedures, and non-specific amplification products lead to false positive results in screening procedures (page 683, second paragraph). The yield and specificity of amplification are improved by another round of amplification (= reamplification)

of the insertion element flanking sequences using additional (nested) primers (Fig. 3; page 683, second paragraph).

Motivation to combine Dellaporta and Koes et al. with Souer et al. Dellaporta teaches amplification of insertion element flanking sequences by iPCR and using the amplified sequences to screen for genes with insertion elements (for example, col. 3, lines 4-24), with the purpose of identifying, as an ultimate goal, novel plants useful to man (col. 36, lines 31-53). Dellaporta stresses the fact that rapid and efficient method of identification of large numbers of insertional mutants are necessary, and provides such method (col. 1, lines 61-63, 66-67; col. 2, line1). In terms of the rapidity and efficiency of the method, amplification of insertion junctions with high yield and specificity are critical to both of these characteristics, since improved amplification yield provides sufficient material for formation of the insertion junction arrays, and improved amplification specificity reduces the number of false positive screening results, leading to identification of potentially useful plants with the minimum of time and expense.

Therefore motivation to combine the references exists in Dellaporta and Souer et al., and the rejection of claim 4 relied on the motivation provided by Souer et al., not on the level of skill in the art, as argued by the Appellants.

Group III (claims 5, 6, 8 and 12): rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta in combination with Koes et al. and Vos et al. (Final Office Action, Paper No. 11, mailed December 19, 2001).

Appellants arguments:

A) References cited do not teach or suggest claim 5, since they do not teach "transposon display amplification". Vos et al. teach a method of DNA fingerprinting, not transposon display amplification (page 10, second paragraph).

B) References cited do not teach the limitations of claim 6, which recites the steps of transposon display amplification. Specifically, Vos et al. does not disclose primers with sequences based on the insertion element sequences (page 10, third paragraph).

C) There is no motivation to combine Dellaporta and Koes et al. with Vos et al. (page 10, the last paragraph, continued on page 11).

D) There would be no reasonable expectation of success to combine the AFLP method of Vos et al. with Dellaporta and Koes et al., because Vos et al. teaches generic primers rather than primers based on the insertion elements (page 11, second paragraph).

Response to Appellants' arguments

Before proceeding with arguments, Appelants' definition of "transposon display amplification" is provided (page 14, lines 7-18):

"Alternatively, the sequences flanking the insertion elements in a specific pool can be recovered using TRANSPOSON DISPLAY amplification. In this case, the DNA samples of the insertion elements mutant library are digested with a restriction enzyme recognizing a site of 6 bases (this restriction enzyme is referred to as the hexacutter) conserved in the insertion element and a restriction enzyme recognizing a site of 4 bases (this restriction enzyme is referred to as the tetracutter). Adaptors are ligated to the obtained fragments and those fragments containing the hexacutter site are selected. Insertion flanking sequences can be amplified in two steps. In the pre-amplification reaction, a primer based on the hexacutter site adaptor and the insertion

element is used in combination with a primer based on the tetracutter site adaptor to amplify a subset of PCR fragments. In the second step, these fragments are used as a template to amplify insertion specific fragments using a transposon specific primer and a primer based on the tetracutter site adaptor (FIG. 2)."

Therefore, transposon display amplification denotes a method comprising the following steps (recited in claim 6):

i) digesting nucleic acid sequences from the gene insertion mutant library with two restriction enzymes, one cutting a 6 base pair (bp) site and the other a 4 bp site,

ii) ligating a biotinylated adaptor to the hexacutter site and a second adaptor to the tetracutter site,

iii) selecting biotinylated restriction fragments using streptavidin beads,

iv) amplifying insertion element flanking sequences using primer based on the biotinylated adaptor and insertion element sequence and a primer complementary to the second adaptor,

v) re-amplifying insertion element flanking sequences using nested primer based on the insertion element and a primer complementary to the second adaptor.

Vos et al. teach a DNA fingerprinting technique comprising the following steps:

i) digesting DNA with two restriction enzymes, recognizing a 6 bp and 4 bp sites, respectively,

ii) ligating an adaptor to the hexacutter site and a second adaptor to the tetracutter site,

iii) selective amplification of the restriction fragments using primers complementary to the adaptors and restriction site sequences.

The amplified fragments could be subjected to a second round of amplification using modified primers (Abstract; page 1408, "AFLP primers and adapters" and "AFLP reactions"). The amplified fragments can be selected by using a biotinylated adapter for the hexacutter site and separated from the rest of the fragments with streptavidin beads (page 4413, col. 2, third paragraph).

Primers consist of three parts: a core sequence and an enzyme specific sequence (which together are complementary to the adapter sequence) and a selective extension, which is complementary to the genomic sequence. The selective extension may consist of one to six nucleotides complementary to genomic DNA (page 1408, the last paragraph; Figure 1; page 4412, first paragraph). Therefore, primers of Vos et al. are not "generic" in a sense that they contain a region complementary to the genomic sequence enclosed between the adapters, which allows selective amplification of sub-sets of sequences. As stated by Vos et al. "The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites.... The method allows the specific co-amplification of high numbers of restriction fragments." (Abstract).

The AFLP method was used for fingerprinting of several genomes, including plant genomes of *Arabidopsis thaliana*, tomato and maize (page 4412, first paragraph; Figure 5).

Vos et al. do not specifically teach amplification of insertion element flanking sequences, but rather their technique is designed to amplify a set of genomic fragments from genomes of

any complexity (page 4413, fifth paragraph). In addition, this method can be applied to pooled DNA samples (page 4414, second sentence). As stated by Vos et al. "In complex genomes the number of restriction fragments that may be detected by the AFLP technique is virtually unlimited. A single enzyme combination (a combination of a specific six-base and four-base restriction enzyme) will already permit the amplification of 100 000s of unique AFLP fragments,..." (page 4413, the last paragraph).

Dellaporta et al. teach that amplification of insertion junctions could be achieved by primer-adapted PCR, which involves restriction with an appropriate restriction enzyme and ligation of primer adapters, followed by amplification of the fragments with a biotinylated primer complimentary to the insertional mutagen. The biotinylated product is purified from the rest of genomic DNA with streptavidin beads and amplified again using adapter-based primer and insertional mutagen specific primer (col. 13, lines 7-16, 31-42). An extra round of amplification of with a nested internal primer (i.e., primer based on the insertion element) and adapter-based primer can be performed to increase specificity.

In summary, Dellaporta teaches elements of the AFLP technique of Vos et al., mainly digestion of the genomic DNA containing insertion element flanking sequences with a restriction enzyme, ligation of adapters and amplification of restriction fragments with both adapter-based and insertion element based primers. Therefore elements of claims 5 and 6 are either taught or suggested by the combination of Dellaporta and Koes et al. with Vos et al.

Motivation to combine the references. Dellaporta teaches amplification of insertion junctions and arrays for the detection of insertional mutants with the purpose of identifying, as an ultimate goal, novel plants useful to man (col. 36, lines 31-53). Dellaporta stresses the fact that

rapid and efficient method of identification of large numbers of insertional mutants are necessary (col. 1, lines 61-63). In terms of the rapidity and efficiency of the method, amplification of insertion junctions in high numbers and with high specificity are critical to both of these characteristics, since large number of amplified junctions provides sufficient material for formation of the insertion junction arrays, and improved amplification specificity reduces the number of false positive screening results, leading to identification of potentially useful plants with the minimum of time and expense. As stated by Vos et al. "In complex genomes the number of restriction fragments that may be detected by the AFLP technique is virtually unlimited. A single enzyme combination (a combination of a specific six-base and four-base restriction enzyme) will already permit the amplification of 100 000s of unique AFLP fragments,..." (page 4413, the last paragraph). Therefore Vos et al. provides a solution to the problem of how to achieve rapid and efficient amplification of a large number of fragments. Vos et al. provide an amplification technique which is applicable to complex genomes, such as plants, and allows amplification of hundreds of thousands of unique restriction fragments, as well as selective amplification of these fragments.

Additional motivation for using the method of Vos et al. is given in the following statement: "The rationale for using two restriction enzymes is the following: (i) The frequent cutter will generate small DNA fragments, which will amplify well and are in the optimal size range for separation on denaturing gels (sequence gels). (ii) The number of fragments to be amplified is reduced by using the rare cutter, since only the rare cutter/frequent cutter fragments are amplified. This limits the number of selective nucleotides needed for selective amplification. ...(iv) Using two different restriction enzymes gives the greatest flexibility in 'tuning' the

number of fragments to be amplified. (v) Large numbers of different fingerprints can be generated by the various combinations of a low number of primers." (page 4409, third paragraph).

Therefore, armed with the disclosure of Dellaporta et al. of primer adapted PCR and the paper of Vos et al., skilled artisan would be motivated to use two restriction enzymes, since this allowed for control of a number of restriction fragments and required a limited number of primers to selectively amplify desired sequences.

Group IV (claims 13-17): rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta in combination with Koes et al. (Final Office Action, Paper No. 11, mailed December 19, 2001).

Appellants' arguments

Neither Dellaporta nor Koes et al. suggest or motivate using of a kit.

Response to Appellants' arguments

Dellaporta teaches that "The ultimate goal of producing an array in accordance with current invention, will be in screening large numbers of individuals or subsets of individuals for detection of an insertional mutant." (col. 15, lines 6-9). As one of the methods for genetic characterization of insertional mutants Dellaporta considers chip-based technologies, in which high densities of oligonucleotides are fixed to solid support (col. 24, lines 33-42). Gene chips were commercially available from "Affymetrix" since 1994 and provided efficient and economical gene analysis tools, since each collection of DNA fragments on a chip could be repeatedly hybridized with different sets of probes, and large numbers of DNA fragments could be probed simultaneously. Therefore it would have been obvious for a skilled artisan to provide

the collection of amplified insertion element flanking sequences on a chip, since such a collection would allow screening a large number of insertional mutants in different plant genomes.

Group V (claims 19 and 21): rejected under 35 U.S.C. 103(a) as being unpatentable over Dellaporta in combination with Koes et al. (Final Office Action, Paper No. 11, mailed December 19, 2001).

Appellants' arguments

A) "Dellaporta does not teach or suggest an insertion element library built into a 3D-array of block, row and column pools as required by claim 19. Rather, Dellaporta is limited to a "2x2 grid, [where] pools of DNA are then prepared from all of the individuals within each column and row... [a]lternatively, pools needn't be used." (*Id.*, Col. 15, lines 66-67 and Col. 16, line 3)." (page 6 and 12).

B) Dellaporta does not teach or suggest "amplifying each of said plurality of insertion element flanking sequences from said block, row and column pools", as claimed by claim 19 (page 6, the last paragraph and page 7, the first paragraph; page 12). According to Appellants, Dellaporta teaches amplification methods which are limited to "using a single primer set [that] may amplify a representative sample of insertion junctions from a particular group of individuals" (Col. 12, lines 6-8), and amplification of a large number of insertional mutants such that there will be a high probability of identifying a mutant for any given locus within the population (Col. 3, lines 54-58).

C) Koes et al. do not teach "amplifying each of said plurality of insertion element flanking sequences from said block, row and column pools", as claimed by claim 19 (page 7, second paragraph, page 12).

D) Dellaporta and Koes et al., neither alone, nor in combination, teach all of the claim limitations of claim 19 (page 7, third paragraph).

E) Combination of Dellaporta and Koes et al. is improper, because there is no motivation to combine the teachings of these two references. "The motivation provided by the Examiner to combine Dellaporta and Koes et al. does not suggest a desirability for combining the references, but merely restates why Koes et al. prefers a "one-step three dimensional screening over three repeated rounds of one-dimensional screening." (page 8, the first paragraph and page 13, second paragraph).

F) Combination of Dellaporta and Koes et al. is improper because Dellaporta teaches away from Koes et al. (page 8, second and third paragraphs and page 13, second paragraph). Specifically, Appellants argue that Dellaporta discourages the use of teachings of Koes et al., since Koes et al. teach "site-selected" approach to identifying insertional mutations, and Dellaporta argues that "site-selected" mutagenesis has had limited success in applications to large-scale genomic investigations.

G) Appellants argue that Dellaporta does not teach screening a gene library organized in at least a two-dimensional array and Koes et al. do not teach or suggest producing labeled amplification products to use as probes to hybridize to a gene library fixed on a solid support.

Summarizing, Appellants argue that neither Dellaporta nor Koes et al. or their combination teaches all of the elements of claim 19 and that combining of Dellaporta and Koes

et al. is improper because there is no motivation to combine the references and Dellaporta teaches away from Koes et al.

B) Regarding claim 21, Appellants argue that neither Dellaporta nor Koes et al. suggest or motivate using of a kit.

Response to Appellants' arguments

Teaching or suggesting all of the elements of claim 19. Claim 19 differs from claim 1 in the last step, "producing a set of labeled amplification products representing said insertion element flanking sequences derived from said block, row and column pools to use as probes to hybridize to a solid support to which a gene library has been fixed as target(s) for hybridization, wherein said gene library is organized in a two-dimensional array". The arguments considering elements taught by in the first two method steps were considered in the discussion of claim 1.

Dellaporta teaches that detection of insertional mutants can be achieved in two ways. In the first one, an array of insertion junctions is produced, and detection is achieved by hybridizing a labeled gene probe to the array (embodiment claimed in claim 1 and discussed above). In a second embodiment an array of genetic sequences (= gene library) is formed, and hybridized with labeled probes of amplified insertion junctions (col. 15, lines 47-57). Dellaporta teaches methods for producing arrays on solid supports (col. 14, lines 7-67; col. 15, lines 1-5). It is well known in the art that arrays are produced in at least a two-dimensional format, i.e., the sequences are arranged in rows and columns on a surface. Dellaporta teaches formation of arrays on nylon filters (col. 32, lines 41-55).

Therefore, Dellaporta in combination with Koes et al. teach all of the elements of claim 19. Arguments regarding combining these two references and motivation for the combination are provided above in the discussion of claim 1.

Teaching or suggesting all of the elements of claim 21. Dellaporta teaches that "The ultimate goal of producing an array in accordance with current invention, will be in screening large numbers of individuals or subsets of individuals for detection of an insertional mutant." (col. 15, lines 6-9). As one of the methods for genetic characterization of insertional mutants Dellaporta considers chip-based technologies, in which high densities of oligonucleotides are fixed to solid support (col. 24, lines 33-42). Gene chips were commercially available from "Affymetrix" since 1994 and provided efficient and economical gene analysis tools, since each collection of DNA fragments on a chip could be repeatedly hybridized with different sets of probes, and large numbers of DNA fragments could be probed simultaneously. Therefore it would have been obvious for a skilled artisan to provide the collection of amplified insertion element flanking sequences on a chip, since such a collection would allow screening a large number of insertional mutants in different plant genomes.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

TS
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